

Effect of *Pimpinella anisum L* Aqueous Extract against Oxidative Stress Induced by Lead Exposure in Young Rats Brain

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ABSTRACT

Pimpinella anisum L is widely used in folk medicine as treatment for neurological disorders. The purpose of this study was to investigate the probably beneficial effect of an oral administration of *Pimpinella anisum L* aqueous extract on oxidative stress induced by earlier lead exposure. Intoxicated rats with 0.2% of lead acetate during gestation and lactation were treated with aqueous extract of *Pimpinella anisum L* at dose of 250mg /kg, 500 mg/kg and 750 mg /kg for 15 days. Brains were removed to assess the antioxidants enzymes in the homogenate such as superoxide dismutase (SOD), glutathione S-transferase (GSST) and total antioxidants in both cerebrum and cerebellum. Further, blood was used to determine glucose level and lead estimation. Our results showed that Lead caused a decrease in the activity of SOD and GSST with a diminution in the concentration of total antioxidant capacity in both cerebrum and cerebellum. Oral administration of *Pimpinella anisum L* at three doses of 250 mg/kg, 500 mg/kg and 750 mg/kg enhanced the activity of the antioxidants enzymes and reduced both glucose and lead level in blood. Phytochemicals investigation demonstrated that aqueous extract possess a moderate antioxidant activity. In conclusion, we can note that *Pimpinella anisum L* aqueous extract may have a beneficial effect on oxidative stress induced by earlier exposure to lead acetate. This effect may be attributed to the phytochemicals compounds present in the extract.

KEYWORDS: *Pimpinella anisum L*, phytochemical analysis, antioxidant activity, heavy metal, antioxidant enzymes cerebrum and cerebellum.

INTRODUCTION

Many mechanisms were suggested to explain the neurotoxicity of lead, but the most probably is that lead exposure caused an imbalance between pro- oxidant and antioxidant balance thus by the excessive generation of free radicals and Reactive oxygen species (ROS). These last are highly reactive to membrane lipids, proteins, DNA and are the major contributing factors for stress injuries and lead to rapid cells changes. Oxidative stress caused consequently enhancement of lipid peroxidation, decreased in the activity of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase [1]. Moreover, lead may damage the membrane entirely by inactivation of the essential thiol group's membrane proteins [2]. Previous studies have demonstrated that rat brain in the early stage of development was more vulnerable to lead toxicity.

Pimpinella anisum L., Apiaceae family, is an annual herb indigenous to the Mediterranean region and Western Asia, reaching from 30 to 50 cm in high with white flower and green to yellow seeds [3]. According to the literature data, *Pimpinella anisum L.*, has been used in the treatment of asthma [4, 5, 6], epilepsy [7] and gastrointestinal disorders [8]. It has been also used as carminative, sedative and antiseptic [9, 10]. Only a few studies have pointed on the neurological effect of *Pimpinella anisum L.*, when compared with the other activities such as antioxidant, antibacterial and anti-inflammatory. The aqueous extract of leaves and stems of anise oil have been reported to postpone the onset of picrotoxin induced seizures attacks in mice [11]. Moreover, application of intraperitoneal anise oil diluted in sesame oil at concentration of 0, 25 to 1 ml/kg enhanced the threshold of PTZ induced chronic convulsions in mice [12]. Further [13] had confirmed the neuroprotective effect of *Pimpinella anisum L* essential oil (PEO). All this studies were undertaken on the base of the traditional medicine, which indicated *Pimpinella anisum L* as infusion to induce tranquilizing effects [14, 15, 16].

In the presence of all this huge data on the hazardous effects of earlier exposure to lead and its impairment on the nervous system, this study was conducted to determine the probably beneficial effects of *Pimpinella anisum L*. aqueous extract at three different doses in young rats intoxicated by lead (Pb) during gestation and lactation.

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MATERIAL AND METHODS

Plant material and aqueous extract preparation:

The dry and ripe seeds of *Pimpinella anisum L.*, were purchased from a local herbs market in Chlef Center (Algeria), and were identified by an expert taxonomist. A voucher specimen (N°.435) was deposited at the herbarium of department of Biology, University of Oran 1 (Ahmed Benbella, Algeria). The seeds of *Pimpinella anisum L* were grinded, and then 100 g of the powder were immersed in 1 L of distilled water on heat for 15 minutes [17]. The aqueous extract was filtered through Whatman paper N°1, and lyophilized (CHRISI, ALPHA 1-2LD, Germany). The yield of extraction was 20, 99%.

Phytochemicals investigations:

The content of total polyphenols was determined by using Folin-Ciocalteu methods [18]. The content of total flavonoids was measured according to [19]. The level of Total tannins in the aqueous extract of *Pimpinella anisum L.*, was determined according to [20].

Antioxidant activities of *Pimpinella anisum L* aqueous extract:

The scavenging activities of the prepared aniseed aqueous extract were estimated by using classical DPPH radical test [21] and reducing power test [22].

HPLC analyses of the aqueous extract:

The HPLC (High Performance Liquid Chromatography) of the aqueous extract was prepared by using C18 column (250×4, 6 mm, 5 μM) with isocratic pump and UV-VIS detector. Analyses were carried out according to [23] with injection volume of 20 μl, flow rate (0,7ml/min), mobile phase was Methanol: Water (70/30) and detection was done at 280 nm. Pure anethole (Sigma) was used as standard for the quantitative analysis.

Animals:

Females Wistar rats (*Rattus norvegicus*) weighing 200 ±30 g were used in this study. All animals were obtained from Department of Biology, University Of Oran 1 (Ahmed Benbella, Algeria). The animals were housed in standard conditions with free access to food and water (12 h light/dark, T° 22±2°C). All procedures performed on rats were approved and conducted in accordance with the National Institute of Health Guide (**Reg. No. 488/160/1999/CPCSEA**). After one week of cohabitation with males, females were divided into two groups: control and intoxicated females that received 0.2% of lead acetate in drinking water during gestation and lactation [24]. At weaning, we formed five groups of pups as follows:

Group C: Control rats (issued from control females) received distilled water.

Group Pb: intoxicated rats with lead (issued from intoxicated females) that received distilled water orally as vehicle solution.

Group Pb + P.A.E 250: intoxicated rats (issued from intoxicated females) that received orally *P.anisum L.* aqueous extract (P.A.E) at dose of 250 mg/kg daily for Two weeks [25].

Group Pb+ P.A.E 500: intoxicated rats (issued from intoxicated females) that received orally *P.anisum L* aqueous extract (P.A.E) at dose of 500 mg/kg daily for Two weeks [25].

Group Pb + P.A.E 750: intoxicated rats (issued from intoxicated females) that received orally *P.anisum L.* aqueous extract (P.A.E) at dose of 750 mg/kg daily for Two weeks [25].

Biochemical analysis:

At the end of the experiment, rats were killed in the morning after 12 hours of fasting. Blood was received in heparin tubes, and then centrifuged 3000 g/20 minutes [26] to obtain plasma, which was used for the determination of blood glucose level. Brain was rinsed by ice saline solution (0.9%), removed and dissected out to obtain two different regions: cerebrum and cerebellum that have been homogenized in ice Tris- HCl buffer (pH =7.2) and centrifuged 10.000 runs /10 minutes at 4°C. The final supernatant was used to determine the activity of superoxide dismutase SOD (Kit Cayman, item N °706002, USA), Total antioxidants (Kits Cayman, item N° 709001, USA), and Glutathione -S- transferase GSST [27].

Determination of Lead (Pb) level in Blood:

After sacrifice, blood was received in hemolytic tubes, then samples were transferred to estimate the concentration of lead in blood by atomic absorption spectroscopy. The analyses were realized in AFAK laboratory (technic Center, Oran, ALGERIA).

Statistical analysis:

All results were expressed as mean ±S.E.M (Standard of Error). The data analysis was carried out by using statistical software: R (Team, 2010; R.D.C : a language and environment for statistical computing), Vienna, Austria (R : foundation for statistical computing retrieved from : <http://www.R.project.org>). Kruskal Wallis test and

the Wilcoxon Rank Sum test were used to examine the level of significance between groups. Value of $p < 0.01$ and $p < 0.05$ were considered as significant.

RESULTS

Phytochemicals screening:

As shown in table (01), the aqueous extract of *Pimpinella anisum L* (1mg/ml) contain a specific amount of total polyphenols (2.38 ± 0.01 mg Eq of Acid Gallic /g of Plant). Whereas total flavonoids were presents in an increased concentration (8.06 ± 0.08 mg Eq of Quercetin /g of Plant) when compared to the other phytochemical constituents of this plant. Tannins were presents in the extract at concentration of 0.40 ± 0.004 mg Eq of Acid Gallic /g of Plant as indicated in table (01).

Reducing Power of *P.anisum L* aqueous extract:

The result of this test is represented in Figure 01, which demonstrated that the reducing power of this plant was dose –dependent manner. The plant extract of *Pimpinella anisum L* possess a weaker reducing power when compared with the ascorbic acid, which exhibited the strongest one.

DPPH Free radical scavenging activity of *P.anisum L* aqueous extract:

The reactivity of *Pimpinella anisum L* aqueous extract was analyzed with DPPH test. The Figure 02 represent that aniseed aqueousextract exhibited an antioxidant capacity which was less that the antioxidant of reference ascorbic acid and more than BHT. Hence, the scavenging capacity was dose –dependent, which means more the concentration of plant extract enhanced, more the activity is strong.

HPLC analyses of *P.anisum L* aqueous extract:

The HPLC analyses of aniseed extract (Figure 03) showed several picks with one main thatpossess retention time of 3.497, which was near to the retention timeof Anethole (3.33). This last, was used as standard to carry out this analyze. From the results, we observed that anethole represented the main compound in the aqueous extract of *Pimpinella anisum L* with a concentration of $3.05 \cdot 10^{-2}$ ng/μl(Result of quantitative analysis).

Biochemical Analyses:

Level of glycaemia and Lead (Pb) in blood:

The results of this dosage are represented in the figure 04, which demonstrated that lead exposure caused a significant increase in the level of glucose in blood of the intoxicated rats comparatively with the control rats. Treatment with *Pimpinella anisum L.*, aqueous extract for 15 days decreased significantly ($p < 0.01$ and $p < 0.05$) the concentration of glucose in blood of the tested rats. This diminution was respectively: Pb+ P.A.E 250 (42.01 ± 0.9 mg/dl), Pb+ P.A.E 500 (30.23 ± 2.24 mg/dl) and for the group Pb+ P.A.E (41.48 ± 0.84 mg/dl).

From table 02, we can see clearly that the most elevated concentration of lead acetate (Pb) was found in the blood of intoxicated group (Pb), when compared with the control group ,which represent the lower Pb level ($p < 0.01$). Whereas in the intoxicated and treated group with *P.anisum L* at 03 doses (250 mg/kg, 500 mg/kg and 750 mg /kg respectively) we observed a significant ($p < 0.05$ and $p < 0.01$) diminution of the concentration of lead in blood.

Antioxidants enzymes:

The level of the antioxidants enzymes measured in the brain (cerebrum and cerebellum) of experimental rats were recorded in the table 03.

Superoxide dismutase (SOD):

The level of SOD in cerebrum showed a significant decrease ($p < 0.05$) in intoxicated group (Pb) when compared to the control group (C). Hence, the oral treatment of intoxicated rats by lead with *Pimpinella anisum L* aqueous extract has increased the level of SOD especially at dose of 500mg/kg ($p < 0.05$). In cerebellum, we observed that lead caused a non-significant decrease of SOD level, whereas of the extract of *Pimpinella anisum L* has enhanced the activity of this enzyme but non-significantly for the doses of 500 mg/kg and 750 mg/kg.

Total antioxidants:

Animals exposed to lead acetate during gestation and lactation showed a non-significant decrease in the level of total antioxidant in both cerebrum and cerebellum. For the treated groups with *Pimpinella anisum L* aqueous extract, we observed an improvement of total antioxidant for the tested doses of 250 mg/kg and 750 mg/kg in cerebrum. Whereas in cerebellum, this improvement was significant in the rats treated with *Pimpinella anisum L* at doses of 500 mg/kg and 750 mg /kg.

Glutathion S-Transferase (GSST):

Rats exposed to lead acetate showed a non-significant decrease in GSST activity in both cerebrum and cerebellum. Whereas for the intoxicated rats then treated with *Pimpinella anisum L* aqueous extract, we observed an increase in the activity of GSST in cerebrum at dose of 750 mg/kg. In contrary for the other doses (250mg/kg and 500 mg/kg), we have observed a decrease in GSST level. For cerebellum, the non-significant improvement of this enzyme was recorded for the doses 500 mg/kg and 750 mg/kg of aniseed aqueous extract.

Table 01: results of phytochemicals screening of *Pimpinella anisum L* aqueous extract.

Aqueous extract of <i>Pimpinella anisum L</i>	
Total polyphenols	2.38 ±0. 01 mg Eq of Acid Gallic / g of Plant.
Total Flavonoids	8. 06 ±0. 08 mg Eq of Quercetin /g of Plant.
Tannins	0. 40±0.004 mg Eq of Acid Gallic /g of Plant.

Mg Eq of acid Gallic: the concentration of polyphenols and tannins in the aqueous extract of *Pimpinella anisum L* is expressed as milligram equivalent of acid Gallic per gram of the dry plant.

Mg Eq of Quercetin: the concentration of flavonoids in the aqueous extract of *Pimpinella anisum L* is expressed as milligram equivalent of quercetin per gram of the dry plant. (All values are expressed as Mean± S.E, n=03)

Table 02 : level of lead (Pb) in Blood of rats measuring by atomic spectroscopic method.

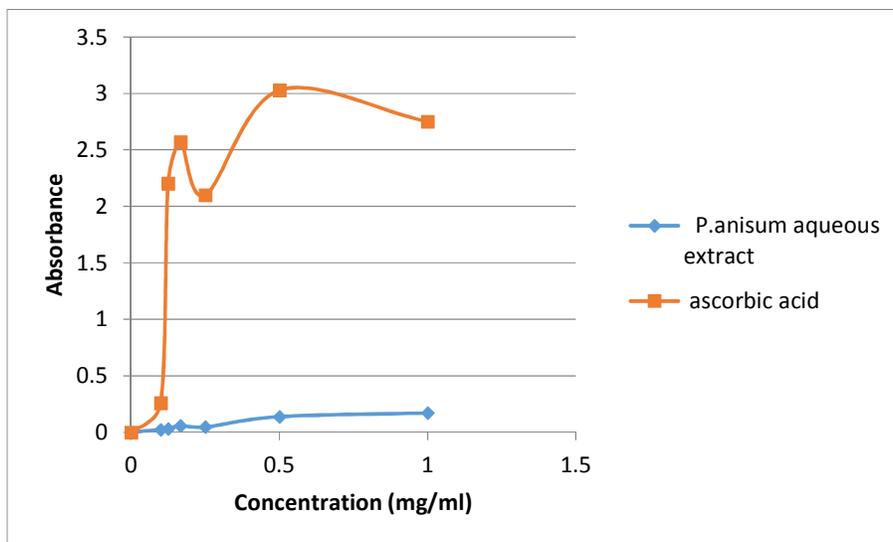
group	Concentration of Pb in blood (µg/L)
C	0.0125±0.0001
Pb	0.251±0.01**
Pb+ P.A.E (250)	0.172±0.0001*
Pb+ P.A.E(500)	0.0807±0.0004**
Pb+ P.A.E(750)	0.0586±0.0001**

(*): $p < 0.05$, (**): $p < 0.01$. (All values are expressed as Mean± S.E, n=08, comparison was done by using Wilcoxon Rank Sum test between: T vs Pb, Pb vs Pb+ P.A.E 250, Pb vs Pb=P.A.E 500 and Pb vs Pb +P.A.E 750)

Table 03: effect of lead acetate (Pb) and *Pimpinella anisum L* aqueous extract (P.A.E) on levels of antioxidants enzymes in cerebrum and Cerebellum.

	Superoxide dismutase (U/mg protein)		Total antioxidants µl/mg protein)		Glutathion S transferase (mmol/min/mg protein)	
	Cerebrum	Cerebellum	Cerebrum	Cerebellum	Cerebrum	Cerebellum
C	0.62±0.034	4.83±0.58	0.266±0.022	2.73±0.23	0.023±0.005	0.20±0.05
Pb	0.46±0.064*	4.59±0.33	0.252±0.028	2.52±0.32	0.013±0.003	0.15±0.04
Pb+ P.A.E(250)	0.48±0.019	2.41±0.18	0.267±0.015	1.03±0.11	0.017±0.007	0.05±0.001
Pb+ P.A.E(500)	0.51±0.017*	5.54±0.29	0.142±0.010	2.91±0.15*	0.010±0.003	0.20±0.07
Pb+ P.A.E (750)	0.49±0.033	5.56±0.31	0.261±0.027	3.51±0.18*	0.012±0.007	0.24±0.09

(*): $p < 0.05$ (All values are expressed as Mean± S.E, n=08, comparison was done by using Wilcoxon Rank Sum test between: T vs Pb, Pb vs Pb+ P.A.E 250, Pb vs Pb=P.A.E 500 and Pb vs Pb +P.A.E 750)

**Figure 01:** Reducing Power activity of *P. anisum L* aqueous extract.

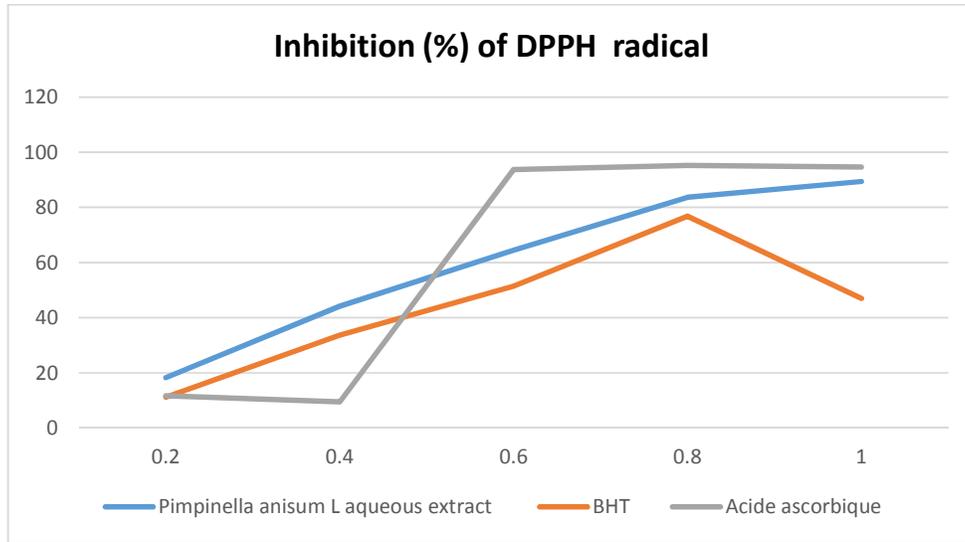


Figure 02: The percentage (%) of DPPH radical inhibition by *Pimpinella anisum L* aqueous extract, BHT and Ascorbic acid.

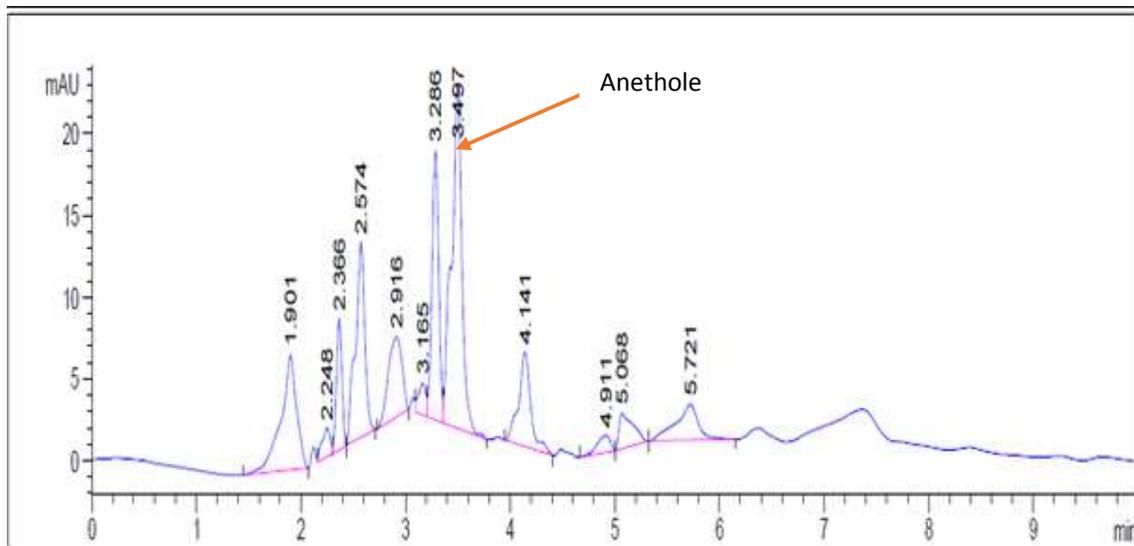


Figure 03: Chromatogram of *Pimpinella anisum L* aqueous extract at concentration of 1 mg/ml obtained by using C18 column, mobile phase: methanol /water (70/30) and UV detector (280 nm).

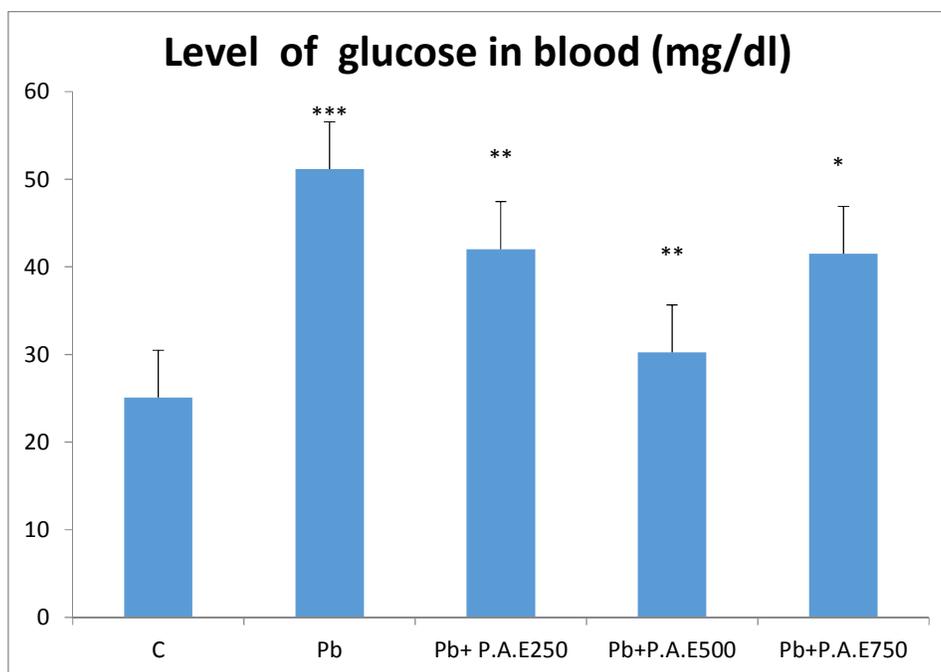


Figure 04 : effect of lead (Pb) and *Pimpinella anisum L* (P.A.E) aqueous extract on the level of glucose in the blood of experimental rats .(All values are expressed as Mean± S.E, n=08, comparasion was done by using Wilcoxon Rank Sum test between: T vs Pb, Pb vs Pb+ P.A.E 250, Pb vs Pb=P.A.E 500 and Pb vs Pb +P.A.E 750)

DISCUSSION

Our study was conducted in the aim of investigate the possible beneficial effect of an oral administration of *Pimpinella anisum L* aqueous extract on oxidative stress induced by prenatal lead exposure. We have started our experiment by phytochemical screening of the studied plant, and our results demonstrated the presence of polyphenols (2.38 ± 0.01 mg Eq of Acid Gallic / g of Plant), flavonoids (8.06 ± 0.08 mg Eq of Quercetin /g of Plant) and Tannins (0.40 ± 0.004 mg Eq of Acid Gallic /g of Plant), which represented the main bioactive compounds. Our findings were in agreement with the previous studies [27, 28]. In general, polyphenols are very important constituents in the plant, but according to the results found by [16], there was no relationship between total phenols and total antioxidant activity in *Pimpinella anisum L* extracts, cause may other factors can play a major role as antioxidants.

In addition, the aqueous extract of aniseed exhibited an antioxidant activity in dose dependent manner. Our result was in agreement with many previous studies, which confirmed that *P.anisum L.*, possess an antioxidant potential [28, 30, 16]. The assay of antioxidant capacity is based on the measurement of scavenging ability of a compound towards the stable radical DPPH. Hence, the scavenging ability of *Pimpinella anisum L* aqueous extract was lower than the reference antioxidant (Ascorbic acid).Moreover, HPLC analyses of *Pimpinella anisum L.*, aqueous extract revealed the presence of Trans anethole as a main compound in the plant extract according to the retention time of the standard used to identify the phytochemical composition of the plant. Our finding agreed with the study of [31].

The second part of our experiment consisted to evaluate the effect of lead and *Pimpinella anisum L* aqueous extract on animals, hence we have recorded that earlier exposure to lead acetate induced biochemical disturbance which was translated by the significance elevation of glucose level in the blood of intoxicated rats. Our results were in accordance with the study of [24] who suggested that lead can induced hormonal stress by interfering with synthesis or section of some hormones. In fact, it has been reported that stress caused an increase of glycaemia under the action of Corticotropin realizing Factor, Corticotropin, cortisone in hypothalamus, hypophysis and surrenal gland respectively by activating enzymes of carbohydrates metabolism [32]. Hence, *Pimpinella anisum L* decreased the level of glucose in intoxicated and treated groups by the three doses (250 mg/kg, 500 mg/kg and 750 mg/kg). According to its antioxidant activity, *Pimpinella anisum L* with their bioactive compounds play an important role in the protection of the body against oxidative stress and free radicals damages, which are the main causes of various metabolic dysfunction [33]. Polyphenols and /or flavonoids can protect body from damages caused by reactive oxygen species (ROS) by acting as chelators agents, binding to transition metals, decomposition of peroxides and free radical scavenging [34].

Lead (Pb) exerts some of its effects by inducing oxidative damages, furthermore a decrease in antioxidant defense mechanisms in the brain was observed in lead exposed animals. The results obtained from our study, demonstrated that lead caused a decrease in the level of SOD, total antioxidants and GSST activity in the brain of intoxicated rats with 0.2% of lead during gestation and lactation. Our finding was in agreement with previous studies [35,36,37,38]. The diminution of SOD level may be explained by the exhaustion of the enzyme itself under stress conditions to make balance between pro-oxidant /antioxidant system [39], cause lead is known to induce free radical damages in tissue by increasing excessive production of reactive oxygen species [40]. The antioxidants enzymes are involved in the defense mechanisms against free radicals threats, hence the observed decrease in the level of total antioxidants in brain confirm that earlier exposure to lead induced depletion of antioxidants enzymes [41]. Our results were in agreement with those of [42], who revealed that lead exposure in 23 days old pups caused decrease in SOD activity.

In fact, [43] reported that nervous system of human and animal is vulnerable to oxidative stress caused by free radical for the following reasons: (1) high concentration of oxidizable substrate in brain like poly – unsaturated fatty acid, (2) low level of antioxidant enzymes in brain and (3) high ratio of membrane surface. Frankly, almost results from this experiment were non-significant, this may be due to the diminution of statistical samples means shorter of sampling but its stay a disputable results.

Only few studies have pointed on the beneficial effect of *Pimpinella anisum L* aqueous extract on neuronal activity especially after lead exposure during earlier life. Anise oil has been investigated for its beneficial effects on memory disorders, depression, cerebral ischemia and Alzheimer diseases [44]. Moreover, Aniseed extract such as essential oil had showed a considerable antioxidants activity and seems to play a contributor role in the protection of the body against the oxidative stress and free radical damage [33].

The results of our study showed that oral administration of *Pimpinella anisum L.*, aqueous extract at three different doses: 250 mg/kg, 500 mg/kg and 750 mg/kg in intoxicated rats by lead acetate during gestation and lactation, have improved the activity of the antioxidant enzymes in cerebrum and cerebellum of experimental rats. This finding was in agreement with previous studies [45], which demonstrated that aniseed increased the activities of antioxidants enzymes such as: superoxide dismutase, catalase and Glutathion peroxidase. Hence, this study had shown that *P. anisum L* aqueous extract exhibited a moderate antioxidant activity and may cause a reversal effect on lead induced changes on the oxidative biomarkers in brain. The observed changes could be attributed to the different polyphenols, flavonoids, tannins and Anethole present in the extract of the studied plant. Moreover, several studies have proved that the extract of aniseed has free radical scavenging and antioxidant properties [16, 46,30].

Conclusion:

From this data, we can conclude that *Pimpinella anisum L* aqueous extract had a moderate beneficial effect on neurotoxicity induced by lead, which was related to the antioxidant activity of the plant. More studies, must be conducted to prove that aqueous extract of *Pimpinella anisum L* had a neuroprotective proprieties on normal and intoxicated rats, beside isolation of the bioactive compound (s) must done to determine its effect separately on animal model.

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